Rapid Host Cell Protein (HCP) Detection with Advanced Trapped Ion Mobility Spectrometry (TIMS) Technology

The biopharmaceutical field is rapidly expanding, with biotherapeutic analysis and bioprocess development seeing particularly significant growth in recent years. The market has a projected growth of 8.59% CAGR from 2019–2024⁴, which will continue to help the industry address the unmet medical needs for the world’s most challenging diseases.

The percentage of large molecules in the pharmaceutical pipeline rose from 30% in 2010 to 42% in 2017⁵ and there are nine Food and Drug Administration (FDA) approved biosimilars on the market, five of which are from 2017. There is, therefore, an ever-increasing requirement for analytical methods with rigorous process-related impurity testing, to monitor these contaminants during manufacture and prior to product release.

During biotherapeutic manufacturing, process-related impurities derived from the host organism can contaminate drug products. For example, during the expression of a recombinant protein drug, host cell systems can express numerous endogenous proteins, known as host cell proteins (HCPs). These contaminants may significantly affect drug efficacy and sometimes cause immunogenicity, making their removal highly important to ensure drug safety and purity. Biotherapeutics, such as monoclonal antibodies (mAbs), are purified using chromatographic techniques designed to remove residual HCP, DNA and viruses, as well as product-related impurities, such as fragments and aggregates. However, low-level HCPs often remain after purification, so the detection and quantification of these residual impurities is critical for biopharmaceutical companies to comply with regulatory guidelines.

Impact of Residual HCPs
There are several ways in which biotherapeutics contaminated with residual HCPs could compromise patient safety. The drug’s efficacy and toxicity can be affected, and the therapeutic window in which the drug acts can be altered. Immunogenicity is the primary concern, where the HCP can invoke an unwanted immune response and cause damage to the patient, even at trace levels. For example, the clinical trial of IXinity (formerly IB1001) for the treatment of haemophiliacs was suspended in 2012, due to the development of antibodies to the drug in 26% of patients³.

The stability of biotherapeutics may also be compromised by the presence of residual HCPs. They can contain enzymes, such as oxidases and lipases, which gradually break down the therapeutic protein or excipient, affecting the stability of the product over time. HCPs are also capable of mimicking the action of therapeutic proteins in assays, possibly resulting in the misformulation of the product outside the therapeutic window ⁴.

Impurity Testing Technologies
Comprehensive HCP analysis allows biopharmaceutical manufacturers to ensure quality control between batches, and facilitates comparisons between biologic drugs and biosimilars. The gold standard technique for HCP analysis is the enzyme-linked immunosorbent assay (ELISA), which is a commonly-used immunoassay for measuring antibodies, antigens, proteins and glycoproteins in biological samples. Despite its high throughput, sensitivity and selectivity capabilities, the ELISA can overlook weak and non-immunoreactive HCPs, which still carry the potential to impact drug safety and efficacy. It is also process-specific, meaning a new assay is required after each process change, which may take several months.

Many biopharmaceutical companies are now looking towards orthogonal methods, such as mass spectrometry (MS), as an alternative or complementary technique for HCP analysis. MS can rapidly monitor and identify multiple protein analytes in the same sample, and detect very low amounts of HCP in a non-targeted manner. This is crucial, considering that even very low-level HCPs can cause immunogenicity and impact drug quality. Whereas an ELISA can only measure the total HCP, MS can provide detailed information on the level of each individual HCP. Advanced MS techniques not only monitor, but characterise several impurities simultaneously, using a single method, bringing the high discriminatory power needed to separate impurities, while providing high sensitivity to detect and quantify low-level HCPs.

Analysing HCPs with MS
Although MS is gaining popularity as a powerful technique, there are still some limitations to overcome. A mass spectrometer with up to six orders of magnitude dynamic range is required to directly detect 1 to 100 ppm of HCPs in the sample, which is out of the range of many current instruments⁶. One way of overcoming the issue of dynamic range for HCP characterisation is to use ion mobility. Some modern instruments use trapped
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ion mobility spectrometry (TIMS) quadrupole time-of-flight (QTOF) MS to achieve high confidence in identification and deep coverage, without compromising speed.

TIMS is a separation technique in gas phase, which resolves sample complexity with an added dimension of separation, in addition to high performance liquid chromatography (HPLC) and MS. This method increases peak capacity and confidence in compound characterisation and, combined with parallel accumulation serial fragmentation (PASEF) technology, enables high sequencing speeds while generating high-quality spectra. The high resolving power of TIMS MS makes it useful for the analysis of complex samples, such as those of biological origin.

PASEF scans implemented on TIMS QTOF MS/MS can combine fast acquisition speeds (>100 Hz MS/MS) with high resolution detectors, for high resolution quantifiable data from a complex sample, in a single run. When coupled with an advanced HPLC system, TIMS QTOF MS/MS with PASEF can detect HCPs using a gradient as short as 21 minutes. PASEF enables almost 100% duty cycle, for maximum use of all ions and therefore superior sensitivity.

There are two approaches to HCP analysis by MS:

1. HCP discovery
This method is useful in early process development, where critical problems arise in production or stability testing. HCP discovery uses longer runs for increased depth of analysis, typically two hours or more. Quantitation is not always necessary, as the primary concern is identifying all the proteins in the mixture. Maximum depth of coverage can be achieved, to identify low-level proteases/lipases that can affect stability, as well as other problematic proteins. Hundreds of peptides can be identified in a single run (Figure 1).

For a 1.5 µg NISTmAb tryptic digest, nanoLC provides a 210-minute gradient for deep coverage of HCPs. Sample preparation can either be a standard tryptic digest of the entire mixture (220 HCPs identified), or a native tryptic digest (using protocol from6), where the mAb is left in its native structure, which provides a better dynamic range (280 HCPs identified).

2. Fast HCP screening
The method provides rapid analysis of downstream processes, with fast runs and high throughput. Quantitation is often necessary. Faster analysis is advantageous for more routine applications, but coverage of 50+ HCPs is still attainable at 1% false discovery rate (FDR) using a 21-minute gradient (Figure 2).

Both methods provide unbiased analysis of proteins, accurate mass measurement and, because of the high resolution of both MS and MS/MS, isotopic fidelity. They also provide collisional cross-section (CCS) values for a new dimension of confidence. These are physical parameters of peptides, often more reproducible than LC retention times. Novel MS instrumentation, such as the timsTOF Pro with PASEF (Bruker Daltonics), is now enabling users to combine HCP discovery and screening in one workflow, and accurately and reproducibly measure CCS values. Highly reproducible CCS values eliminate the need to include retention times (RT) in confidence measurements, enabling easy changing of LC methods, columns, flows, and other instrument parameters.

In instances that require deeper coverage of HCPs, samples can be analysed using a standard proteomics setup using nano ultra-high-performance liquid chromatography (UHPLC).

The Future of HCP Analysis
The low abundance of HCPs continues to present a challenge to the biopharmaceutical industry, and work on the removal of impurities has traditionally required specialised setups. PASEF scans implemented on advanced TIMS TOF MS instrumentation...
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The depth of HCP identification provided by PASEF technology allows fingerprinting of biomanufacturing processes and ability to easily identify the effects of changes in these procedures. Sensitive, rapid, in-depth techniques for HCP analysis are driving the field of biotherapeutics, which is now transitioning into a new phase, thanks to decreased run times conferred by PASEF implemented on TIMS TOF MS. Such technologies are enabling biopharmaceutical manufacturers to accurately identify and quantify HCPs as CQAs and, therefore, comply with increasingly stringent regulatory guidelines.

REFERENCES

1.  BIOPHARMACEUTICALS MARKET – GROWTH, TRENDS, AND